REMARKS

Claims 1-80 are in the case. In response to a requirement to restrict, Applicants elected Group IV, drawn to a method for conditionally activating a transgene in a second generation plant and corresponding to Claims 36-43, 70 and 80. Claims 1-35, 44-69, and 71-79 were canceled as drawn to a non-elected invention in a previous response.

Claims 36-38 have been canceled in favor of new claims 81-86. Basis for the new claims is found in the claims as originally filed.

The Claims are rejected variously under 35 U.S.C. §112, and §103.

The claims have been amended to more clearly define Applicants' invention. The marked-up versions of these claim additions are found on a separate sheet attached to this amendment and is titled "Version with Markings to Show Changes Made".

No new matter has been added.

Paragraph numbers used below correspond to those of the pending Office Action.

Election/ Restriction

1. Non-elected subject matter has been removed from Claims 70 and 80.

Priority

2. In compliance with the conditions of 35 U.S.C. §120 for receiving the benefit of an earlier filing date, Applicants have amended the first sentence of the specification on page 1.

Information Disclosure Statement

3. Acknowledgement is made of the requirements of 37 CFR §1.98(b) and the review of the IDSs submitted on 25 January 2001 and 16 April 2001.

Drawings

4. Applicants note the Notice of Draftsperson's Patent Drawing Review (form PTO-948). Applicants will provide formal

drawings with the appropriate corrections at the time of the receipt of the notice of allowability, as provided under 37 CFR §1.85.

Claim Objections

5. Claim 38 is objected to for minor informalities. The Claims have been amended to overcome this objection.

Rejection under 35 U.S.C. §112, Paragraph 2

6. Claims 36-43 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicant regards as the invention. Specifically, the Examiner argues that:

a.) In claims 36-39, the preamble is inconsistent with part 4) of each of the claims;

b.) In claims 37 and 39, it is unclear as to whether or not all three recombinase elements must be located in the same construct (as indicated in part 1);

c.) In claim 37, part 2), it appears that the transgenic plant should comprise the first, rather than the third recombinase element, to maintain consistency with part 4) of the claim; and

d.) In claim 39, the third recombinase element does not clearly state whether it comprises only one or both of the general structures mentioned in part 1c).

Claims 36-38 have been deleted in favor of new Claims 81-86; specifically, new Claims 81 and 82 are derived from Claim 36, new Claims 83 and 84 are derived from Claim 37, and new Claims 85 and 86 are derived from Claim 38. Additionally, Applicants have amended Claim 39 for clarity. In light of these claim amendments, the Applicants respectfully request removal of these rejections.

Rejection under 35 U.S.C. §112, Paragraph 1

7. Claims 36-38 and 43 stand rejected under 35 U.S.C. §112, first paragraph as failing to clearly teach one skilled in the art how to use the claimed invention and for lack of

written description. Specifically, the Examiner argues that while the specification is enabling for a method for conditionally activating a transgene in a second generation plant when the promoter of the third recombinase element is not active in the common germline (using combinations of two site-directed recombination systems to cause developmentally staggered site-specific recombinations to control transgene expression), the specification is not enabling when the third recombinase promoter is active in the germline. Applicants respectfully traverse.

Claims 36-38 have been deleted in favor of new Claims 81-86. In Claims 81, 83, and 85, transgene expression is enabled in a first generation plant, since the only restriction for the nature of the promoters is such that: 1.) P1, P2 and P3 are operably linked to their down stream elements; and 2.) the temporal expression specificity of each promoter is such that the activation of P2-R2 occurs concomitantly with or after P1-R1 and the activation of P3-TG occurs concomitantly with or after P2-R2. Thus, it is possible that P3 is active in the common germline in Claims 81, 83, and 85.

In contrast, in Claims 82, 84, and 86, transgene expression in enabled in a second generation plant, since the P1, P2 and P3 promoters are restricted such that: 1.) P1, P2, and P3 are operably linked to their down stream elements; 2.) P1 and P2 are germline promoters; and, 3.) the temporal expression specificity of each promoter is such that the activation of P2:R2 occurs concomitantly with or after P1:R1 in the first generation common germline cells and the activation of P3:TG occurs in the second generation. Thus, in these instances, P3 may be a germline promoter, but the activation of P3:TG will only occur in the second generation plant and not in the first generation germline cells.

Support for new Claims 81-86 is found in the specification on page 35, lines 4-12:

> "Such combinations of two or more different site-specific recombinations, whether linked or unlinked, provide novel and useful tools to control transgene expression and/or removal in the first, second, or third generations that are not currently available in agricultural biotechnology. Thus, a pair of developmentally staggered SSRs may be used

as ON-ON or ON-OFF (transgene removal) switches. The salient feature in both schemes, is that expression of R2 and/or trait genes does not have to occur immediately upon enablement (i.e., removal of the stop fragment) by R1 but are rather controlled solely by the choices of P2 and P3 promoters."

Further, see page 23, lines 17-22:

"Any promoter functional in a plant will be suitable including but not limited to constitutive plant promoters, plant tissue-specific promoters, plant development-specific promoters, inducible plant promoters, viral promoters, male germline-specific promoters, female germline-specific promoters, flower-specific promoters, and vegetative shoot apical meristem-specific promoters."

Applicants submit that the claims as now amended are fully enabled by the specification. Specifically, Applicants submit that the specification does indeed teach combinations of two site-directed recombination systems which cause developmentally staggered site-specific recombinations to control expression of a transgene in a plant, both when the third recombinase promoter is not active in the germline and when the third recombinase promoter is active in the germline.

In light of the amendments to the claims Applicants request removal of these rejections and reconsideration of the claims.

Rejection under 35 U.S.C. §103

8. Claims 36-43, 70, and 80 are rejected under 35 U.S.C. §103(a) as being unpatentable over Odell et al. "A" (*Mol. Gen. Genet.* 223: 369-378 (1990)), in combination with Lloyd et al. (*Mol. Gen. Genet.* 242: 653-657 (1994)), the present state of the art, and Odell et al. "B" (Use of Site-Specific Recombination Systems in Plants, in Homologous Recombination and Gene Silencing in Plants; Paszkowski, J., Ed.; Kluwer: Dordrecht, Germany, 1994; pp 219-270). Applicants respectfully traverse this rejection and submit that this combination of references fails to establish a *prima facie* case of obviousness.

- Odell et al. "A" teach the use of the Cre/lox recombination system in transgenic tobacco and propose that turning on expression of a marker gene by

controlling Cre expression in a regulated manner would provide the ability to follow cell lineages in the plant.

- Lloyd et al. teach the use of the FLP/FRT site-specific recombination system in stably transformed tobacco plants.
- The present state of the art teaches specific promoters (including seed specific promoters, common germline promoters, floral common germline promoters, and inducible promoter systems) and various transgenes.
- Odell et al. "B" teach uses of various site-specific recombination systems individually in plants. It is asserted that the versatility and high recombination frequency of these systems allow their use as tools for a wide range of studies and applications and that the systems can be used to control gene expression in any plant generation and/or tissue.

The Examiner argues that it would have been obvious to one skilled in the art to combine the teachings concerning single site-specific recombination systems (Odell et al. A and Lloyd et al.) with the teachings of specific promoters and transgenes (Applicant's admitted state of the prior art), and further combine these teachings with the teachings of the wide range of applications of single site-specific recombination systems (Odell et al. B) to derive the present invention.

It is well settled that to establish a *prima facie* case of obviousness three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations [MPEP 706.02(J)].

Whether there is a reasonable expectation of success is a function of the predictability of the art at the time the invention was made in view of the knowledge of the person of skill in the art [MPEP 2143.02].

Applicants argue that the combination of the above cited references do not render the claims obvious as the skilled person would have no reasonable expectation of success in obtaining the present invention by combining Odell et al. A, Lloyd et al., Applicant's admitted state of the prior art, and Odell et al. B.

First, Odell et al. A and Lloyd et al. teach only single site-specific recombination (using the Cre/lox system and the FLP/FRT system, respectively), and thus do not teach all of the limitations of the invention as claimed. They do not teach combinations of two or more site-specific recombinase elements or methods of using multiple site-specific recombinase elements. Nor is there a suggestion in either Odell et al. A or Lloyd et al. that it would be useful to combine more than one site-specific recombinase element to control the timing of expression of a transgene in a plant.

Secondly, Odell et al. A, Odell et al. B, and Lloyd et al. (singly or in combination) do not suggest the novel applications that result by introduction of multiple site-specific recombinase elements into a plant, and thus the skilled person would have no reasonable expectation of success in deriving the present invention from the teaching in the art. While single site-specific recombinase elements serve as a single one-way gene switch to turn genes 'ON' or 'OFF' (transgene removal), linked site-specific recombination (i.e., whereby one site-specific recombinase element activates the recombinase of another site-specific recombinase element) can serve as either an 'ON-OFF' or a 'ON-ON' switch. This allows novel applications that are not possible with single site-specific recombinase elements. One example of these novel applications is trait expression only in the second generation. The prior art is silent as to whether both multiple, linked site-specific recombinase elements and their novel applications. Thus a person of skill in the art, at the time the invention was made, would not have a reasonable expectation of success in developing a system utilizing multiple site-specific recombinase elements. At best, the skilled person might find it obvious to try to use two or more site-specific

recombinase elements; however, "obvious to try" is not the standard by which a *prima facia* case of obviousness under 35 U.S.C. §103 may be sustained [MPEP 2145 X].

Finally, Applicant's submit that until Applicants' demonstration for the utility of use of multiple, linked site-specific recombinase elements, the skilled person would not have been motivated to look to the prior art for the selection of regulated germline promoters.

In summary, none of the reference relied upon anticipate or render obvious the claimed invention and Applicant respectfully requests reconsideration of Claims 36-43, 70, and 80 as amended.

In view of the foregoing arguments and the amendments made to the Claims, Applicants respectfully request removal of all rejections and reconsideration of the Claims.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In showing the changes, deleted material is shown as strikethrough, and inserted material is shown underlined.

IN THE SPECIFICATION:

Please amend the paragraph beginning on page 1, line 5 as follows:

~~This application is a continuation in part of USSN 09/442,021, filed November 17, 1999,~~ which claims the benefit of U.S. Provisional Application 60/063504, filed October 24, 1997

IN THE CLAIMS:

Please cancel Claim 36-38 in favor of new Claims 81-86.

Please add new Claims 81-86 as follows:

--81. A method for conditionally activating a transgene in a plant comprising:

1) providing constructs comprising:

a) a first recombinase element having the general structure P1-R1;

b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;

c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

(i) P1 is a first promoter;

(ii) R1 is a first recombinase coding sequence and 3' region;

(iii) RS1 is a first recombinase site responsive to a first recombinase;

(iv) P2 is a second promoter;

(v) RS2 is a second recombinase site responsive to a second recombinase;

(vi) STP is a stop fragment;

(vii) R2 is a second recombinase coding sequence and 3' region;

(viii) TG is a transgene sequence and 3' region; and

(vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and
wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, and the activation of P3, driving expression of TG, occurs concomitantly with or after P2, driving expression of R2;

2) providing a transgenic plant comprising the first, second and third recombinase elements;

3) activating P1 such that the R1 recombinase coding sequence is expressed in a first generation plant, wherein expression of R1 excises the stop fragment from the second recombinase element;

4) activating P2 such that R2 is expressed, wherein expression of R2 excises the stop fragment from the third recombinase element allowing expression of the transgene in the first and all subsequent generations of plants.--

--82. A method for conditionally activating a transgene in a second generation plant comprising:

1) providing constructs comprising:

a) a first recombinase element having the general structure P1-R1;

b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;

c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

(i) P1 is a first germline promoter;

(ii) R1 is a first recombinase coding sequence and 3' region;

(iii) RS1 is a first recombinase site responsive to a first recombinase;

(iv) P2 is a second germline promoter;

(v) RS2 is a second recombinase site responsive to a second recombinase;

(vi) STP is a stop fragment;

(vii) R2 is a second recombinase coding sequence and 3' region;

(viii) TG is a transgene sequence and 3' region; and

(vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and

wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, in the first generation common germline cells and the activation of P3, driving expression of TG, occurs in the second generation;

2) providing a transgenic plant comprising the first, second and third recombinase elements;

5) activating P1 such that the R1 recombinase coding sequence is expressed in the common germline of a first generation plant, wherein expression of R1 excises the stop fragment from the second recombinase element;

6) activating P2 such that R2 is expressed in the common germline of the first generation plant, wherein expression of R2 excises the stop fragment from the third recombinase element allowing expression of the transgene in the second and all subsequent generations of plants.--

--83. A method for conditionally activating a transgene in a plant comprising:

1) providing constructs comprising:

a) a first recombinase element having the general structure P1-R1;

b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;

c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

(i) P1 is a first promoter;

(ii) R1 is a first recombinase coding sequence and 3' region;
(iii) RS1 is a first recombinase site responsive to a first recombinase;
(iv) P2 is a second promoter;
(v) RS2 is a second recombinase site responsive to a second recombinase;
(vi) STP is a stop fragment;
(vii) R2 is a second recombinase coding sequence and 3' region;
(viii) TG is a transgene sequence and 3' region; and
(vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, and the activation of P3, driving expression of TG, occurs concomitantly with or after P2, driving expression of R2;

2) providing a transgenic plant comprising the third recombinase element;
5) transforming the transgenic plant of (2) with either the first recombinase element to generate a first plant or the second recombinase element to generate a second plant;
6) crossing the first and second plants such that expression of R1 is expressed and excises the stop fragment from the second recombinase element allowing expression of R2 under the control of P2 which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 in the first and subsequent generation(s).--

--84. A method for conditionally activating a transgene in a second generation plant comprising:

1) providing constructs comprising:

a) a first recombinase element having the general structure P1-R1;

b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;

c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

(i) P1 is a first germline promoter;

(ii) R1 is a first recombinase coding sequence and 3' region;

(iii) RS1 is a first recombinase site responsive to a first recombinase;

(iv) P2 is a second germline promoter;

(v) RS2 is a second recombinase site responsive to a second recombinase;

(vi) STP is a stop fragment;

(vii) R2 is a second recombinase coding sequence and 3' region;

(viii) TG is a transgene sequence and 3' region; and

(vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and

wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression if R1 in the first generation common germline cells and the activation of P3, driving expression of TG, occurs in the second generation;

2) providing a transgenic plant comprising the third recombinase element;

3) transforming the transgenic plant of (2) with either the first recombinase element to generate a first plant or the second recombinase element to generate a second plant;

4) crossing the first and second plants such that expression of R1, under the control of P1 in the common germline of the first generation, excises

the stop fragment from the second recombinase element allowing expression of R2 under the control of P2 in the common germline of the first generation plant which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 in the second and subsequent generation(s).--

--85. A method for conditionally activating a transgene in a plant comprising:

1) providing constructs comprising:

a) a first recombinase element having the general structure P1-R1;

b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;

c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

(i) P1 is a first promoter;

(ii) R1 is a first recombinase coding sequence and 3' region;

(iii) RS1 is a first recombinase site responsive to a first recombinase;

(iv) P2 is a second promoter;

(v) RS2 is a second recombinase site responsive to a second recombinase;

(vi) STP is a stop fragment;

(vii) R2 is a second recombinase coding sequence and 3' region;

(viii) TG is a transgene sequence and 3' region; and

(vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, and the activation of P3, driving

expression of TG, occurs concomitantly with or after P2, driving expression of R2;

2) providing a transgenic plant comprising the first, second and third recombinase elements;

3) inducing the first promoter such that R1 is expressed under the control of P1 in the first generation, wherein R1 excises the stop fragment from the second recombinase element allowing expression of R2 under the control of P2, which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 promoter in the first and subsequent generation(s).--

--86. A method for conditionally activating a transgene in a second generation plant comprising:

1) providing constructs comprising:

a) a first recombinase element having the general structure P1-R1;

b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;

c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

(i) P1 is a first germline promoter;

(ii) R1 is a first recombinase coding sequence and 3' region;

(iii) RS1 is a first recombinase site responsive to a first recombinase;

(iv) P2 is a second germline promoter;

(v) RS2 is a second recombinase site responsive to a second recombinase;

(vi) STP is a stop fragment;

(vii) R2 is a second recombinase coding sequence and 3' region;

(viii) TG is a transgene sequence and 3' region; and

(vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and
wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, in the first generation common germline cells and the activation of P3, driving expression of TG, occurs in the second generation;

2) providing a transgenic plant comprising the first, second and third recombinase elements;

3) inducing the first promoter such that expression of R1, under the control of P1 in the common germline of the first generation, excises the stop fragment from the second recombinase element allowing expression of R2 under the control of P2 in the common germline of the first generation plant, which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 promoter in the second and subsequent generation(s).

Please Amend the following claims as indicated:

39. (Amended One Time) —A method for conditionally activating a transgene in a ~~second generation~~ plant comprising:

1) providing ~~a~~ constructs comprising:

a) a first recombinase element having the general structure P1-R1;

b) a second recombinase element the having general structures P2-RS1-STP-RS1-R2;

c) a third recombinase element ~~is selected from the group consisting of~~having the general structures P3-RS2-STP-RS2-TG1; and

d) a fourth recombinase element having the general structure P4-RS2-STP-RS2-TG2;

wherein:

(i) P1 is a first promoter;

(ii) R1 is a first recombinase coding sequence and 3' region;

(iii) RS1is a first recombinase site responsive to a first recombinase;
(iv) P2 is a second promoter;,
(v) RS2 is a second recombinase site responsive to a second recombinase;
(vi) STP is a stop fragment;
(vii) R2 is a second recombinase coding sequence and 3' region;
(viii) TG1 is a first transgene sequence and 3' region;
(ix) TG2 is a second transgene sequence and 3' region;
(ix) P3 is a third promoter; and
(x) P4 is a fourth promoter;

wherein P1, P2, P3 and P4 are operably linked to their down stream elements and wherein TG1 and TG2 are different trait transgenes and wherein P3 and P4 are activated in a second generation plant;

2) providing a first and second plant selected from the group consisting of:
a) a first plant comprising the first and third recombinase elements, and a second plant comprising the second and fourth recombinase elements;
b) a first plant comprising the first and fourth recombinase elements and a second plant comprising the second and third recombinase elements;

~~3) providing a second plant comprising the second and third recombinase elements;~~

34) crossing the first and second plants to produce a first generation plant wherein conditional expression of the first recombinase coding sequence (R1) under the control of the P1 promoter in the common germline of the first generation, excises the stop fragment from the second recombinase element allowing ~~expressing~~ expression of the second recombinase coding

sequence and 3' region (R2) under the control of P2 promoter, which recombinase, in turn, excises the stop fragments from the ~~two~~ third and fourth recombinase elements, permitting expression of the trait gene(s) TG1 and TG2 under the control of P3 and P4 promoter, respectively, in the second generation.

70. (Amended One Time) A method for the conditional expression ~~or excision~~ of a transgene in a plant comprising:

(i) providing a multiplicity of recombinase elements, each recombinase element comprising:

a) at least one promoter;

b) a DNA fragment;

wherein the DNA fragment comprises at least one genetic element selected from the group consisting of:~~,~~ a recombinase coding sequence, a site-specific recombinase sequence responsive to a recombinase, a stop fragment and a transgene;

(ii) introducing at least two of the recombinase elements of step (i) into at least one plant wherein the at least two recombinase elements are selected from the group consisting of:

a) a recombinase element having a first recombinase under the control of a promoter; and

b) a recombinase element having a second recombinase under the control of a promoter whose expression is dependent on the expression of the first recombinase;

(iii) activating ~~at~~ the promoter of step (ii)(a) wherein the expression of the second recombinase is effected by the expression of the first recombinase.

80. (Amended One Time) A trait expression ~~removal~~ construct comprising:

a) a first recombinase element comprising a first promoter operably linked to a sequence encoding a first recombinase;

b) a second recombinase element comprising a second promoter, a stop fragment bounded by site specific sequences responsive to the first recombinase and a sequence encoding a second recombinase wherein the presence of the stop fragment inhibits expression of the second recombinase, and wherein the first and second recombinases are different; and

c) a DNA molecule bounded by site specific sequences responsive to the second recombinase;

wherein expression of the first recombinase excises the stop fragment from the second recombinase element, operably linking the second promoter and the sequence encoding the second recombinase, and wherein expression of the second recombinase results in site specific recombination within the DNA molecule bounded by site specific sequences responsive to the second recombinase.